



# Effects of long-term fencing on soil microbial community structure and function in the desert steppe, China

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**Abstract:** One of the goals of grazing management in the desert steppe is to improve its ecosystem. However, relatively little is known about soil microbe communities in the desert steppe ecosystem under grazing management. In this study, we investigated the diversity and aboveground biomass of *Caragana korshinskii* Kom. shrub communities in long-term fencing and grazing areas, combined with an analysis of soil physical-chemical properties and genomics, with the aim of understanding how fence management affects plant-soil-microbial inter-relationships in the desert steppe, China. The results showed that fence management (exclosure) increased plant diversity and aboveground biomass in *C. korshinskii* shrub area and effectively enhanced soil organic carbon (233.94%), available nitrogen (87.77%), and available phosphorus (53.67%) contents. As well, the Shannon indices of soil bacteria and fungi were greater in the fenced plot. Plant-soil changes profoundly affected the alpha- and beta-diversity of soil bacteria. Fence management also altered the soil microbial community structure, significantly increasing the relative abundances of Acidobacteriota (5.31%–8.99%), Chloroflexi (3.99%–5.58%), and Glomeromycota (1.37%–3.28%). The soil bacterial-fungal co-occurrence networks under fence management had higher complexity and connectivity. Based on functional predictions, fence management significantly increased the relative abundance of bacteria with nitrification and nitrate reduction functions and decreased the relative abundance of bacteria with nitrate and nitrite respiration functions. The relative abundances of ecologically functional fungi with arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and saprotrophs also significantly increased under fence management. In addition, the differential functional groups of bacteria and fungi were closely related to plant-soil changes. The results of this study have significant positive implications for the ecological restoration and reconstruction of dry desert steppe and similar areas.

**Keywords:** desert steppe; fence management; *Caragana korshinskii*; soil physical-chemical property; soil microorganism

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## 1 Introduction

Desert steppe (the transition zone from a typical steppe to a desert) is an important ecosystem, unique to Eurasia, and has a long history of livestock grazing (Kang et al., 2007; Zhang et al.,

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2018). In the desert steppe ecosystem with relatively simple plant species richness and community structure, high-intensity grazing exacerbates the environmental pressure on plant community (Herrero-Jáuregui and Oesterheld, 2018; Gao and Carmel, 2020). In order to alleviate this threat and mitigate the negative impacts on grassland ecosystem, government in China has initiated a series of ecological protection policies and restoration projects since the late 1990s and early 2000s. For example, in 2003, the Returning Pasture to Grassland project was implemented, which uses a system of grazing bans and rotational grazing to reduce grazing pressure on natural grasslands (Xiong et al., 2016; Xu et al., 2016). Subsequently, the Grassland Ecological Conservation project was implemented in 2011 to mitigate grassland degradation and increase grassland vegetation biomass (Hao et al., 2014). The implementation of these ecological engineering projects has had a positive impact on the structure of plant communities and the physical and chemical properties of soil, contributing to the restoration of the grassland ecosystem (Li et al., 2017; Sun et al., 2023).

Researchers generally agree that improving grazing management system is the principal strategy for the revegetation and reconstruction of arid and semi-arid grassland ecosystem (Xu et al., 2018). Limiting selective foraging by herbivores directly alters the availability of light to subcanopy species, which, in turn, affects the structure and composition of plant community (Sun et al., 2023). However, studies have shown that the response of species richness to fencing is not consistent. For example, long-term fencing of grasslands in the Mu Us Sandy Land, China increased vegetation cover and the Shannon-Wiener diversity index, but decreased plant species richness (Liu et al., 2019). In shrubby grassland ecosystem of Inner Mongolia Autonomous Region, China, long-term fencing was found to increase Shannon-Wiener diversity and species richness (Hao et al., 2019). The responses of plant community diversity and richness to fencing in desert grassland ecosystem dominated by xerophytic shrubs deserve further investigation.

In the desert steppe ecosystems, the physical and chemical properties of soil have long been important factors influencing vegetation cover. The trampling of vegetation by livestock has been shown to destroy the soil surface crust and aggregates and increase soil erodibility (Munkhtsetseg et al., 2017). The loss of soil nutrients is further exacerbated by external forces, such as wind (Aubault et al., 2017). In addition, the degradation of vegetation induced by grazing reduces the quantity and quality of aboveground plant litter input, which adversely affects the physical and chemical properties of soil (Penner and Frank, 2019). The implementation of fencing usually increases vegetation cover in arid and semi-arid grassland ecosystem, reduces evapotranspiration, and facilitates water storage in soil (Mekuria and Veldkamp, 2012). The removal of animal feces and urine can also effectively reduce soil pH (Fan et al., 2020). Furthermore, fencing protects the aboveground and belowground parts of plants and further enhances plant-soil interactions, which not only facilitates the accumulation of soil organic carbon but also effectively improves the accumulation of soil nutrients, such as available nitrogen and phosphorus (Zheng et al., 2023).

It is well known that soil microorganisms are an important part of ecosystem and an important medium for the transfer of nutrients and information between plants and environment (Faghihinia et al., 2020). Studies have shown that changes in plant community structure and soil physical-chemical properties as a result of fencing also affect the structure and function of soil microbial community (Aubault et al., 2015; Zhang et al., 2016). For example, fencing significantly increases the abundance of soil bacteria and fungi in desert grasslands (Fan et al., 2020), and alters the relative abundances of Actinobacteria, Proteobacteria, and Ascomycota (Zhu et al., 2021). Additionally, the effects of fencing on soil moisture, pH, and nutrients create ecological niches for underground microbial community (Yao et al., 2018). Microbial network relationships can further explain this change. More stable and connected network relationships are conducive to the occupation of ecological niches by bacteria and fungi and play a crucial role in maintaining the stability of microbial communities (da Costa et al., 2021; Fu et al., 2022). For example, fencing increases the efficiency of soil microbial nitrogen utilization, reduces soil N<sub>2</sub>O loss (Zhang et al., 2021), and increases the relative abundance of functional fungal groups (Eldridge and Delgado-Baquerizo, 2018). However, the responses of soil bacterial and fungal

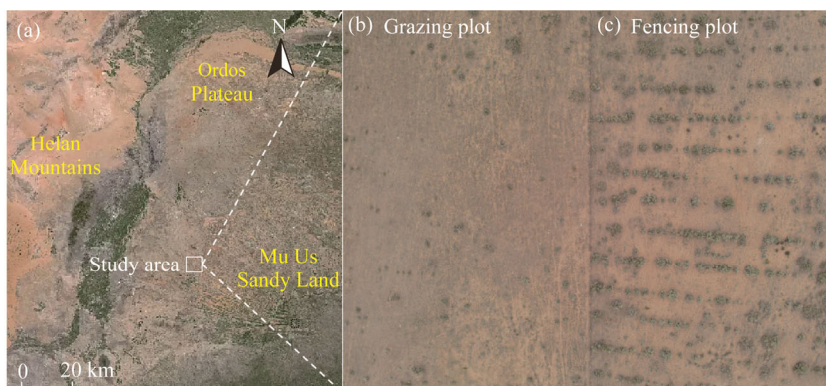
functional groups to fencing in desert grasslands have not been sufficiently investigated.

In many studies of grassland management, the focus has been on the synergistic changes and responses of plant-soil microorganisms under different grazing regimes. In view of the importance of soil microbial communities in ecosystem restoration, we focused our attention on the *Caragana korshinskii* Kom. shrub area of the Ordos Plateau, northern China, an area between the Helan Mountains to the west and Mu Us Sandy Land to the east. *C. korshinskii* is the dominant species for the ecological restoration of desert grasslands and has the characteristics of wind prevention and drought resistance (Chen et al., 2022). Through the investigation of plant communities, analysis of soil physical and chemical properties, and results of bacterial 16S ribosomal ribonucleic acid (rRNA) gene and internal transcribed spacer (ITS) high-throughput sequencing, the following questions were addressed: (1) whether changes in plant community structure and soil physical-chemical properties under fence management positively affect ecological niche competition among soil microorganisms; and (2) how potential functions of soil bacteria and fungi respond to fence management.

## 2 Materials and methods

### 2.1 Study area and experimental design

The study area was located in the Otag Front Banner, Inner Mongolia Autonomous Region, China (37°43'–37°55'N, 107°21'–107°38'E; 1300–1350 m a.s.l.; Fig. 1) with a typical temperate continental climate. The sampling time was in late June 2021, the planting time of *C. korshinskii* was approximately 2004, and the fences were installed in 2014. Nine study plots (5 m×5 m) were placed inside and outside the fenced enclosures, and the samples were spaced more than 50 m apart. The diversity and richness of plants were assessed using the per-tree survey method. The plants studied included *C. korshinskii*, *Achnatherum splendens* (Trin.) Nevski, *Stipa glareosa* P.A. Smirn., *Lespedeza potaninii* Vass., *Cynanchum komarovii* Al., and *Sophora alopecuroides* L.



**Fig. 1** Study area (a) and study plots (b and c) in the Otag Front Banner, Inner Mongolia Autonomous Region, China

### 2.2 Soil sample collection

Soil samples were collected in August 2022. Within the study area, nine grazing plots (5 m×5 m) and nine fencing plots (5 m×5 m) were divided, of which we further divided five sub-samples in each quadrat based on five-point sampling method, and mixed as one soil sample (Fig. S1). A total of 18 soil samples were obtained. The sampling depth was 0–20 cm, and the samples were taken after removing litter from the surface and avoiding plant roots (Pan et al., 2021). These collected soil samples were immediately placed in an icebox and returned to the laboratory (Kang et al., 2022). These fresh soil samples were sent to Novogene Bioinformatics Technology Co., Ltd., Beijing, China and stored at low temperature for the determination of soil bacteria and fungi.

### 2.3 Analysis of soil physical-chemical properties

Air-dried soil was used to determine soil physical-chemical properties. The soil samples were sieved and weighed to determine soil water content (SWC). Ten grams of soil was weighed in a beaker, 50 mL of deionized water was added, a conductivity meter (DDS-307A, LEICI Scientific Instruments Co., Ltd., Shanghai, China) was used to determine the soil electrical conductivity (EC), and a pH meter (PHS-3C, LEICI Scientific Instruments Co., Ltd., Shanghai, China) was used to determine soil pH (Bao, 2000). Soil samples were air-dried and an Elementar Vario Macro cube was used to determine total carbon (TC) and nitrogen (TN) contents in the soil. Total phosphorus (TP) was measured using the alkaline potassium persulfate digestion method (Bettinelli et al., 2000). Soil organic carbon (SOC) content was determined using the dichromate oxidation method, available nitrogen (AN) content was determined using the alkali hydrolysis method, and available phosphorus (AP) content was determined using the ammonium molybdate colorimetric method (Bao, 2000; Bettinelli et al., 2000).

### 2.4 Polymerase chain reaction (PCR) amplification and sequence processing

Soil deoxyribonucleic acid (DNA) was extracted from 18 soil samples using the hexadecyl trimethyl ammonium bromide (CTAB) method. The soil bacterial V3-V4 gene region and fungal internal transcribed spacer-1 (ITS-1) were amplified using primers (341F, 806R, ITS1F, and ITS2) (Claesson et al., 2009; Cheng et al., 2022). After PCR amplification, the products were extracted from a 2% agarose gel and purified for quantification (Pan et al., 2021; Kang et al., 2022). We used the NEXTflex™ Rapid DNA-Seq Kit (Bioo Scientific, Austin, USA) to construct the PE (paired-end) library and sequenced it using a NovaSeq PE250 platform. Trimmomatic method was used for quality control of the raw MiSeq data, which was then merged with fast length adjustment of short reads (FLASH) v.1.2.11 (Wang et al., 2007). UPARSE v.7.0.1090 was used to perform operational taxonomic unit (OTU) cluster analysis on the sequence (similarity=97%) (Edgar, 2013). The representative sequence of OTU was obtained by removing chimeras during the clustering process. The species classification annotation of each sequence was performed using ribosomal database project (RDP) classifier and compared with the Silva database (SSU128) using a confidence threshold of 0.7 (Quast et al., 2013). The 16S rRNA and ITS gene sequences obtained in this study were submitted to NCBI (National Center for Biotechnology Information) SRA (Sequence Read Archive) database with serial numbers PRJNA1026058 and PRJNA1026303, respectively.

### 2.5 Data analysis

Plant community indices, including Shannon-Wiener ( $H$ ), Simpson ( $H'$ ), Pielou ( $E$ ), and Patrick ( $R$ ) indices (Shannon, 1948; Zhang et al., 2003) were calculated using the following equations:

$$H = - \sum_{i=1}^S P_i \ln P_i, \quad (1)$$

$$H' = 1 / \sum_{i=1}^S P_i^2, \quad (2)$$

$$E = H / \ln S, \quad (3)$$

$$R = S, \quad (4)$$

where  $P_i$  is the relative importance value of the  $i^{\text{th}}$  species; and  $S$  is the number of species.

The above-ground biomass (AGB; kg) estimation in this study was performed using the method described by Conti et al. (2019).

$$\text{AGB} = e^{-2.281 + 1.525 \ln BD + 0.831 \ln CD + 0.523 \ln HP}, \quad (5)$$

where  $BD$  is the ground diameter (cm);  $CD$  is the crown breadth (cm); and  $HP$  is the plant height (cm).

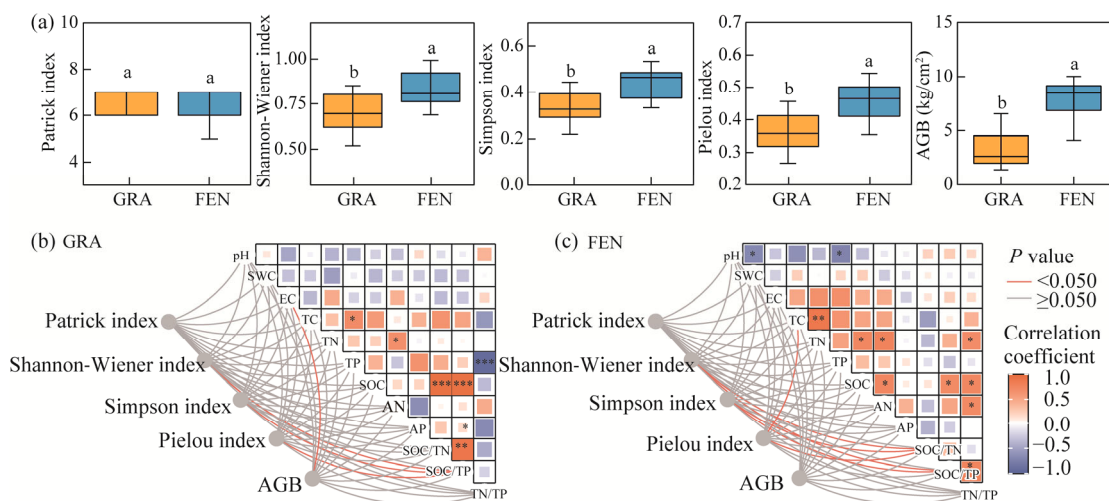
Mantel's test was used to describe the correlation between plant community indices and soil

physical-chemical properties (Sunagawa et al., 2015). Quantitative insights into microbial ecology (QIIME) v.1.9.1 software was used to analyze the microbiome alpha-diversity (OTUs, Shannon, and richness) (Caporaso et al., 2012). Non-metric multidimensional scaling analysis (NMDS) was used to analyze the beta-diversity of bacterial and fungal communities (Bell et al., 2014). The phyla (relative abundance > 1%) of the microbial community were analyzed using the "ggalluvial" package in the R software program (Pan et al., 2021). The relative abundances of bacterial and fungal phyla were analyzed using Spearman's test ( $P < 0.05$ ) (Kang et al., 2022). We calculated Spearman's coefficients for the OTUs of bacteria and fungi under different plots ( $|r| > 0.9$ ,  $P < 0.001$ ) and then displayed the co-occurrence network with established data (e.g., showing the number of nodes in Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, and Ascomycota) using Cytoscape software v.3.7.1, and data on nodes and edges were obtained (Kang et al., 2022). Additionally, we calculated the network parameters for each OTU for subsequent analyses. We used redundancy analysis (RDA) to assess the effects of fence management on soil physical-chemical properties and soil microbial communities. Subsequently, the biogeochemical functions of bacterial communities were predicted using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) (Louca et al., 2016). Fungi+Functional+Guild (FUNGuild) has also been used to predict the ecological functions of fungal communities (Nguyen et al., 2016). The bacterial and fungal functional groups between the two plots were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests ( $P < 0.050$ ). Finally, Spearman's analysis showed the correlation of plant community indices, soil physical-chemical properties, and differential functional taxa (Pan et al., 2022).

## 3 Results

### 3.1 Effects of fencing on plant community structure and soil physical-chemical properties

Fencing increased Shannon-Wiener, Simpson, and Pielou indices of *C. korshinskii* communities in the desert steppe and significantly increased plant AGB, but had no significant effect on the Patrick index (Fig. 2a). Fencing also decreased soil pH and EC, but had little effect on SWC. Further analysis revealed that fencing significantly increased soil TC, TN, and TP contents, with



**Fig. 2** Plant community indices (a) of *Caragana korshinskii* shrub and their correlation with soil physical-chemical properties of the GRA (b) and FEN (c) plots in the desert steppe. GRA, grazing; FEN, fencing. In Figure 1a, different lowercase letters indicate significant differences between the two plots at  $P < 0.050$  level. Lines in the box are median values. Bars are standard errors. \*,  $P < 0.050$  level; \*\*,  $P < 0.010$  level; \*\*\*,  $P < 0.001$  level. AGB, above-ground biomass; SWC, soil water content; EC, electrical conductivity; TC, total carbon; TN, total nitrogen; TP, total phosphorus; SOC, soil organic carbon; AN, available nitrogen; AP, available phosphorus. The abbreviations are the same in the following figures.

more pronounced increases in SOC, AN, and AP contents (Table 1). In addition, fencing significantly increased SOC/TN and SOC/TP ratios. Mantel's test showed that Shannon-Wiener, Simpson, and Pielou indices had significant correlation with SOC/TP ratio in the grazing plot, respectively, whereas AGB was significantly related to EC ( $P<0.050$ ; Fig. 2b). In the fencing plot, Shannon-Wiener, Simpson, and Pielou indices were significantly correlated with SOC/TN ratio as well as SOC/TP ratio ( $P<0.050$ ; Fig. 2c).

**Table 1** Soil physical-chemical properties of the GRA and FEN plots in the desert steppe

Soil physical-chemical property	GRA	FEN
pH	8.84±0.02 <sup>a</sup>	8.65±0.01 <sup>b</sup>
SWC	13.87±0.56 <sup>a</sup>	14.41±0.64 <sup>a</sup>
EC (μS/cm)	74.37±2.57 <sup>a</sup>	64.08±1.62 <sup>b</sup>
TC (g/kg)	4.23±0.22 <sup>b</sup>	9.31±0.50 <sup>a</sup>
TN (g/kg)	0.28±0.005 <sup>b</sup>	0.40±0.025 <sup>a</sup>
TP (g/kg)	0.12±0.005 <sup>b</sup>	0.22±0.012 <sup>a</sup>
SOC (g/kg)	0.63±0.05 <sup>b</sup>	2.11±0.12 <sup>a</sup>
SOC/TN	2.82±0.16 <sup>b</sup>	5.33±0.22 <sup>a</sup>
SOC/TP	5.41±0.38 <sup>b</sup>	9.61±0.62 <sup>a</sup>
TN/TP	2.40±0.11 <sup>a</sup>	1.81±0.11 <sup>b</sup>
AN (mg/kg)	6.22±0.37 <sup>b</sup>	11.68±0.99 <sup>a</sup>
AP (mg/kg)	1.26±0.08 <sup>b</sup>	1.94±0.14 <sup>a</sup>

Note: GRA, grazing; FEN, fencing; SWC, soil water content; EC, electrical conductivity; TC, total carbon; TN, total nitrogen; TP, total phosphorus; SOC, soil organic carbon; AN, available nitrogen; AP, available phosphorus. The abbreviations are the same in the following tables. Different lowercase letters within the same row indicate significant differences between two plots at  $P<0.050$  level; Mean±SE.

### 3.2 Effects of fencing on soil microbial diversity

The number of OTUs and Shannon indices of bacteria and fungi in the soil increased under fencing, but the richness index was not significantly different ( $P<0.050$ ; Fig. 3a and b). NMDS analyses showed that the stress values of bacterial and fungal communities were less than 0.2000, indicating that there were between-group differences in both bacterial and fungal communities in both the grazing and fencing plots, and that the differences in soil fungal communities were more pronounced in the two plots (Fig. 3c and d).

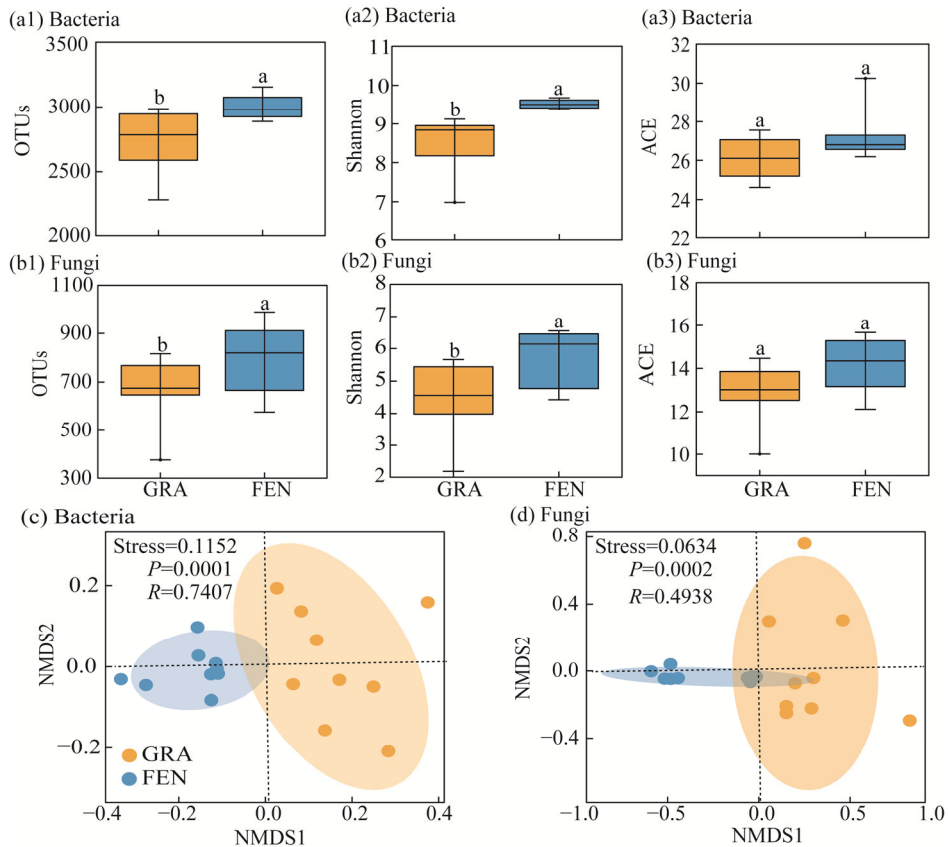
### 3.3 Effects of fencing on soil microbial community structure

In the fencing and grazing plots, 84.36% of the OTUs were annotated at the phylum level, with Actinobacteriota, Proteobacteria, Acidobacteriota, and Firmicutes as the dominant phyla in all samples. Among the fungi, 70.33% of the OTUs were annotated at the phylum level, with Ascomycota, Basidiomycota, Mortierellomycota, and Glomeromycota being the dominant phyla in all samples (Fig. 4a and b). Fencing significantly increased the relative abundances of Acidobacteriota (5.31%–8.99%) and Chloroflexi (3.99%–5.58%), whereas it decreased the relative abundance of Proteobacteria (25.34%–15.24%). However, fencing also significantly increased the relative abundance of Glomeromycota (1.37%–3.28%) (Fig. 4c and d).

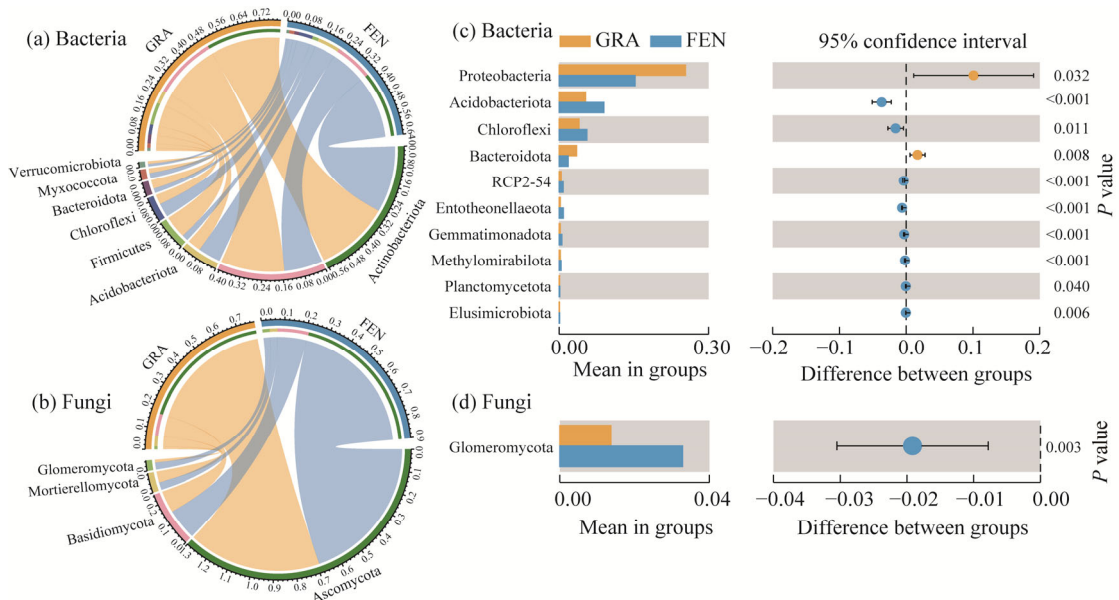
### 3.4 Effects of fencing on soil bacterial-fungal network relationships

In both two plots, the OTUs of Actinobacteriota, Proteobacteria, Acidobacteriota, Chloroflexi, and Ascomycota exhibited large ecological niches in the network (>5% of the nodes). In the grazing plot, the number of nodes of Actinobacteria and Proteobacteria in the network was higher than in the fencing plot (Fig. 5a and b). The grazing plot had 1311 nodes and 2926 edges, with 2216 positive and 710 negative edges. The fencing plot contained 1715 nodes and 3706 edges, with 1965 positive and 1741 negative edges. In addition, the average path length and graph diameter parameters of the bacterial-fungal co-occurrence network were also higher in the fencing plot than in the grazing plot (Table 2).

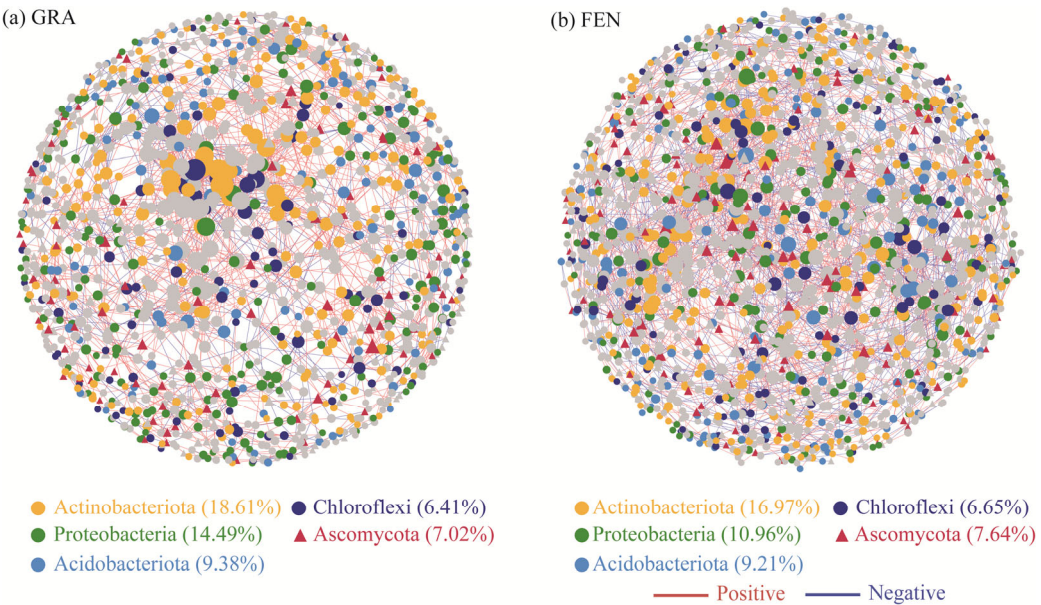




**Fig. 3** Alpha- and beta-diversity of soil bacterial (a1–a3 and c) and fungal (b1–b3 and d) communities of the GRA and FEN plots in the desert steppe. In Figure 3a and b, different lowercase letters indicate significant differences between the two plots at  $P < 0.050$  level. Lines in the box are median values. Bars are standard errors. OTUs, operational taxonomic units; ACE, abundance-based coverage estimator; NMDS, non-metric multidimensional scaling.



**Fig. 4** Relative abundance of bacteria (a) and fungi (b) greater than 1% at the phylum level, and the difference of relative abundance of bacteria (c) and fungi (d) of the GRA and FEN plots in the desert steppe. Bars are standard errors.



**Fig. 5** Co-occurrence network of soil bacteria and fungi of the GRA (a) and FEN (b) plots in the desert steppe

**Table 2** Network topological features of the GRA and FEN plots in the desert steppe

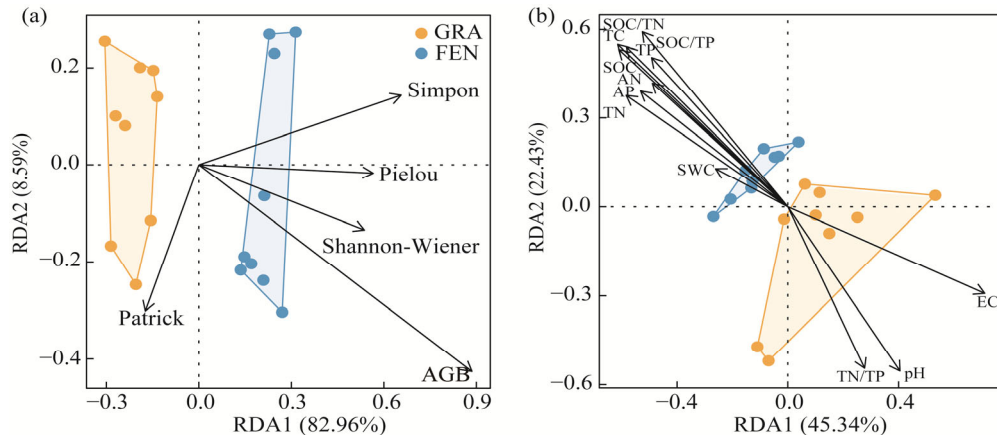
Network parameter	GRA	FEN
Node	1311	1715
Edge	2926	3706
Positive edge	2216	1965
Negative edge	710	1741
Number of modules	201	187
Modularity	0.76	0.77
Average path length	7.32	7.51
Graph diameter	18.42	21.33
Clustering coefficient	0.33	0.33
Degree centralization	0.015	0.007

**3.5 Effects of fencing on soil bacterial and fungal functions**

RDA of plant community indices and soil physical-chemical properties revealed that RDA1 explained 82.96% of the soil physical-chemical properties, and AGB had a greater effect on soil physical-chemical properties ( $P=0.005$ ; Fig. 6). Further RDA of soil microbial community by soil physical-chemical properties revealed that RDA1 explained 45.34% of the soil microbial community, and all the factors except SWC and TN/TP ratio, had a strong correlation on soil microbial community ( $P<0.050$ ; Fig. 6b).

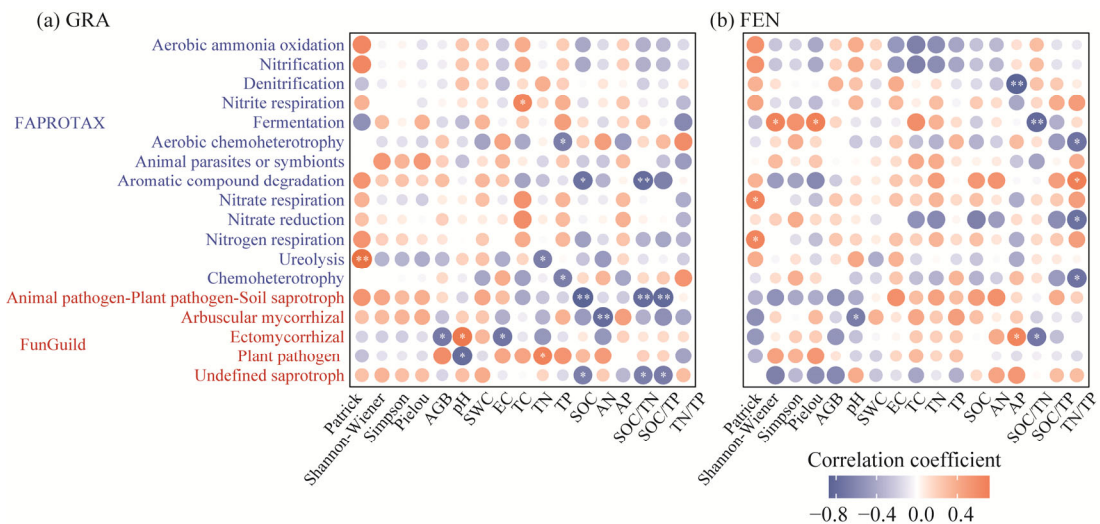
Functional predictions of bacterial (FAPROTAX) and fungal (FUNGuild) communities showed significant differences in nitrogen cycle functional taxa (e.g., aerobic ammonia oxidation, nitrification, denitrification, nitrite respiration, and fermentation) between grazing and fencing bacterial communities; whereas saprotroph fungi and ectomycorrhizal were the dominant functional taxa of fungal communities (Table S1). Through a FAPROTAX prediction of soil bacterial function, we found that fencing significantly increased the relative abundance of bacteria with nitrification and nitrate reduction functions, while decreasing the relative abundance of bacteria with nitrate and nitrite respiration functions. FUNGuild predictions showed that the relative abundance of fungi with ecological functions, such as arbuscular mycorrhizal and





**Fig. 6** RDA (redundancy analysis) of plant community indices (a) and soil physical-chemical properties (b) of the GRA and FEN plots in the desert steppe

ectomycorrhizal fungi, increased under fencing. In addition, we analyzed the differential functions of bacteria and fungi with plant community indices and soil physical-chemical properties in the two plots, and in the grazing plot, SOC, SOC/TN and SOC/TP ratios were negatively correlated with pathogen-saprotroph taxa (Fig. 7a). In the fencing plot, TN/TP ratio showed negative correlations with aerobic chemoheterotrophy and chemoheterotrophy ( $P < 0.050$ ; Fig. 7b).



**Fig. 7** Spearman's correlation of differential functioning of soil bacteria and fungi with soil physical-chemical properties of the GRA (a) and FEN (b) plots in the desert steppe. \*,  $P < 0.050$  level, \*\*,  $P < 0.010$  level.

## 4 Discussion

### 4.1 Soil nutrient accumulation in the desert steppe under fencing

Fencing is a major driver of plant community structure and function in the desert steppe (Ye et al., 2023). The results of our study showed that fencing significantly increased Shannon-Wiener, Simpson, and Pielou indices, and AGB compared with those under grazing (Fig. 2). It has been shown that fencing increases plant Shannon-Wiener and Simpson indices and effectively improves the biodiversity of desert areas (Tang et al., 2016). Zhang et al. (2018) pointed out that grazing intensity was negatively correlated with AGB, and helped to maintain higher species diversity and productivity in the desert steppe of northern China. The results of the present study

confirmed the previous findings and also showed that pH and EC were significantly lower in the fencing plot compared with those of the grazing plot (Table 1). Li et al. (2014) noted that reduced AGB due to grazing increased surface water evaporation (resulting in surface salt accumulation), which in turn increased soil pH and EC. In addition, the accumulation of herbivorous urine and feces has been reported to increase soil pH (Li et al., 2014). Notably, Shannon-Wiener, Simpson, and Pielou indices were significantly correlated with SOC/TP ratio in both grazing and fencing plots (Fig. 2b and c). Under the background of limited soil phosphorus, the input of plant litter causes the increase of SOC content (Deng et al., 2014; Bai et al., 2020). In this study, the effect of fencing on the biomass of plant species in the desert steppes is one of the most important factors affecting the physical properties of soil.

Findings from several studies demonstrate that an increase in plant AGB after fencing, as well as in litter and root exudates, contributes to soil carbon and nitrogen accumulation (Wu et al., 2014). Similarly, in the present study, soil in the fencing plot had higher TC and TN contents compared with those in the grazing plot (Table 1). Wang et al. (2016) found that soil carbon and nitrogen stocks changed most significantly in the surface layer (0–30 cm) after fencing and pointed out that fencing is an effective measure for improving carbon and nitrogen pools of the desert steppe ecosystems. It is well known that grassland productivity in northern China is constrained by soil phosphorous content (Lu et al., 2023). In our study, fencing was found to increase soil TP content (Table 1). A meta-analysis also indicated that fencing could increase TP content of grassland in arid and semi-arid areas of northern China (Wang et al., 2018). Fencing can improve carbon, nitrogen, and phosphorous contents in the surface layer of desert steppe soils.

SOC is a major source of soil nutrients, and fencing can directly affect SOC content by influencing the accumulation of AGB (Wilson et al., 2018). These findings are further supported by the fact that SOC content was more significantly associated with AGB in our study and that fencing significantly increased SOC content (Table 1). In addition, fencing also increased the contents of AN and AP, and it has been noted that soil TN and AN contents were not only significantly correlated after long-term fence management but also effectively increased soil TN and AN contents (Zhu et al., 2016). There are conflicting reports in the literature; in some cases, short-term fencing had less of an effect on soil AN. However, in the present study, we report results from a long-term (20 a) enclosure experiment, which may explain the more pronounced effect on AN. Another explanation is that *C. korshinskii* belongs to the leguminous family and thus has a stronger biological N fixation capacity than common ordinary forage. As a result, plant growth was further promoted in the absence of grazing, which improved the amount of AN in the soil (Doll, 2023). Overall, fencing is an effective measure for increasing grassland productivity and improving soil fertility, particularly in the arid and semi-arid desert steppes of northern China.

#### 4.2 Soil microbial community structure in the desert steppe under fencing

In our study, fencing significantly increased the diversity of soil bacterial and fungal communities (Fig. 3). It has been demonstrated that ecological restoration of grasslands by fencing increases the diversity of soil microbial bacteria and fungi along with plant diversity and biomass (Hu et al., 2016). In addition, there were differences between soil bacterial and fungal communities in both the two plots, which have been observed in other study (Wang et al., 2022). Further analysis found that the dominant groups of soil microorganisms in the desert steppe are Proteobacteria, Acidobacteria, Bacteroidetes, Firmicutes, Ascomycota and Basidiomycota (Fan et al., 2021; Kang et al., 2023). Our study not only revealed changes in the relative abundance of these dominant phyla but also found that Acidobacteriota has a high explanatory rate in the desert steppe. Zhang et al. (2021) and Li et al. (2022) pointed out that fencing could increase the relative abundance of Acidobacteria in the soil and was positively correlated with SOC, TN, AK, and AP.

The response characteristics of soil microbial communities to environmental changes have been visualized using co-occurrence networks (de Vries and Wallenstein, 2017). We constructed soil bacterial-fungal co-occurrence networks for the grazing and fencing plots and found that fencing

had more complex network relationships. The number of nodes and edges in the fencing plot was higher than in the grazing plot, and the average path length and graph diameter were also higher than those in the grazing plot. Previous studies have found that fencing increases the complexity of soil bacterial and fungal networks (Jing et al., 2023), which plays a crucial role in maintaining the stability of microbial communities (Fu et al., 2022). We further analyzed and found that the nodes and edges of the bacterial-fungal co-occurrence network were higher than under fencing than under grazing, with higher network complexity and connectivity. More studies have indicated that increased grazing intensity reduces the complexity of soil bacterial-fungal co-occurrence networks; more stable and connected network relationships favor improved plant production performance (da Costa et al., 2022). Therefore, soil bacterial-fungal network relationships can also be an important basis for evaluating fence management in the desert steppe.

### 4.3 Function of soil microbial community in the desert steppe under fencing

Soil microorganisms play key roles in energy flow and material circulation in ecosystems (Veresoglou et al., 2015). In our study, FAPROTAX predictions showed that fencing significantly increased the relative abundance of bacteria with nitrification and nitrate reduction functions, while decreasing the relative abundance of bacteria with nitrate and nitrite respiration functions. Similarly, a recent study by Zhang et al. (2023) found that grazing increased the relative abundance of soil bacteria with denitrification functions. It is worth noting that there was a correlation between SOC/TN ratio, SOC/TP ratio, and nitrogen cycle taxa in the fencing plot, which may be caused by the effect of increased SOC content on denitrification processes (Zhang et al., 2021). Additional studies have also pointed out the effects of grazing on the relative abundance of bacteria with nitrogen cycling functions, which may lead to a loss in soil nutrient availability (Olsen et al., 2011; Pan et al., 2018). Combined with plant communities and soil physical-chemical properties, it is clear that fencing has a positive effect on soil nitrogen cycling and accumulation.

In this study, FUNGuild predicted that the relative abundance of fungi with ecological functions, such as arbuscular mycorrhizal and ectomycorrhizal fungi, could be enhanced by fencing (Table S1). Arbuscular mycorrhizal fungi are ubiquitous and specialized symbiotic fungi belonging to Glomeromycota (Šmilauer et al., 2021). According to Figure 4, we found that the relative abundance of Glomeromycota was significantly higher in the fencing plot than in the grazing plot, which may be an important reason for the increase of arbuscular mycorrhizal and ectomycorrhizal fungi groups. In view of the important role of mycorrhizal fungi in above- and below-ground multi-nutrient cycling, it can be further inferred that the relative abundance of mycorrhizal fungi groups increases by fencing, thus indirectly enhancing soil nutrient cycling (Fei et al., 2022; Chen et al., 2023). In addition, the relative abundance of saprotrophic fungi increased significantly by fencing in this study. Previous studies have reported that an increase in SOC increases competition by saprophytic fungi groups (Kang et al., 2023). Our study further demonstrated the effect of fencing on the potential relationship between saprotrophic fungi groups and soil physical-chemical properties.

## 5 Conclusions

In the context of increased disturbances from grazing, excluding livestock through fencing can restore and protect plant community structure and soil physical-chemical properties in the desert steep ecosystems. As shown by the results, soil microbial community structure and function respond positively to fencing. The study demonstrated that fencing improved soil by increasing AGB levels in the soil of *C. korshinskii*. In addition, long-term fencing resulted in marked enhancement of SOC, AN, and AP contents, as well as increased Shannon indices of bacteria and fungi in the soil. Plant-soil changes profoundly affect the alpha- and beta-diversity of soil bacteria. The exclusion of grazing also altered the soil microbial community structure, significantly

increasing the relative abundances of Acidobacteriota, Chloroflexi, and Glomeromycota. The soil bacterial-fungal co-occurrence networks under fencing had higher complexity and connectivity. RDA demonstrated the role of AGB in influencing the desert steppe soil environment and the importance of SOC, AN, and AP contents for soil microbial communities. Based on functional predictions, fencing significantly increased the relative abundance of bacteria with nitrification and nitrate reduction functions and decreased the relative abundance of bacteria with nitrate and nitrite respiration functions. The relative abundances of ecologically functional fungi with arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and saprotrophs also significantly increased under fencing. In addition, the differential functional groups of bacteria and fungi are closely related to plant-soil changes. The comprehensive results of this long-term study offer a solid basis for the ecological restoration and reconstruction of the dry desert steppes.

## Conflict of interest

LI Xinrong is an editorial board member of Journal of Arid Land and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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## Author contributions

Conceptualization: PAN Yaqing, KANG Peng, LI Xinrong; Methodology: PAN Yaqing, KANG Peng, QU Xuan; Investigation: PAN Yaqing, KANG Peng, QU Xuan, RAN Yichao; Formal analysis: KANG Peng, RAN Yichao; Writing - original draft preparation: PAN Yaqing, KANG Peng; Writing - review and editing: PAN Yaqing, LI Xinrong; Funding acquisition: KANG Peng, LI Xinrong. All authors approved the manuscript.

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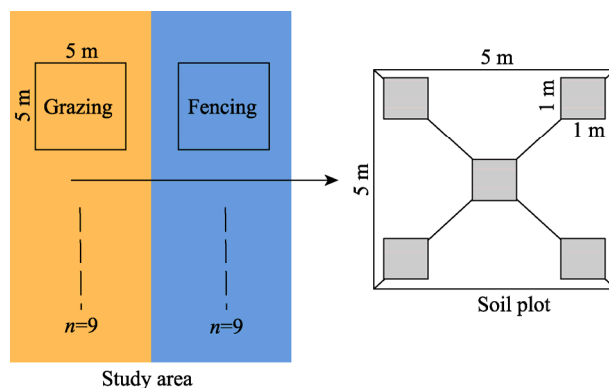
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## Appendix



**Fig. S1** Sampling diagram of *Caragana korshinskii* Kom. shrub in the desert steppe

**Table S1** Differential functional taxa of bacterial and fungal communities of the GRA and FEN plots in the desert steppe

Functional taxa	GRA		FEN		<i>P</i> -value	<i>Q</i> -value	Interval lower limit	Interval upper limit
	Mean	SD	Mean	SD				
Aerobic ammonia oxidation	0.0099	0.0076	0.0349	0.0162	0.0014	−0.0279	−0.0380	−0.0118
Nitrification	0.0099	0.0076	0.0349	0.0162	0.0014	−0.0279	−0.0380	−0.0119
Denitrification	0.0012	0.0009	0.0003	0.0002	0.0219	−0.0279	0.0002	0.0016
Nitrite respiration	0.0023	0.0011	0.0008	0.0008	0.0040	−0.0279	0.0006	0.0025
Fermentation	0.0096	0.0042	0.0058	0.0014	0.0273	−0.0279	0.0005	0.0070
Aerobic chemoheterotrophy	0.1611	0.0526	0.1103	0.0078	0.0200	−0.0279	0.0103	0.0914
Human pathogens septicemia	0.0001	0.0001	0.0000	0.0035	0.0433	−0.0279	0.0003	0.0002
Animal parasites or symbionts	0.0066	0.0031	0.0034	0.0019	0.0232	−0.0279	0.0005	0.0059
Plant pathogen	0.0001	0.0001	0.0002	0.0026	0.0206	−0.0279	0.0002	0.0002
Aromatic hydrocarbon degradation	0.0003	0.0090	0.0069	0.0042	0.0077	−0.0279	0.0001	0.0003
Aromatic compound degradation	0.0081	0.0042	0.0044	0.0007	0.0266	−0.0279	0.0006	0.0070
Aliphatic non methane hydrocarbon degradation	0.0002	0.0093	0.0063	0.0037	0.0027	−0.0279	0.0005	0.0002
Hydrocarbon degradation	0.0003	0.0086	0.0069	0.0042	0.0041	−0.0279	0.0001	0.0003
Nitrate respiration	0.0026	0.0011	0.0010	0.0007	0.0021	−0.0279	0.0007	0.0026
Nitrate reduction	0.0229	0.0070	0.0424	0.0107	0.0004	−0.0279	−0.0287	−0.0105
Nitrogen respiration	0.0028	0.0009	0.0010	0.0007	0.0002	−0.0279	0.0010	0.0026
Ureolysis	0.0105	0.0062	0.0029	0.0014	0.0064	−0.0279	0.0027	0.0123
Chemoheterotrophy	0.1751	0.0599	0.1161	0.0074	0.0183	−0.0279	0.0128	0.1051
Animal pathogen-Plant pathogen-Soil saprotroph-Undefined saprotroph	0.0002	0.0002	0.0016	0.0014	0.0223	0.0530	−0.0024	−0.0003
Arbuscular mycorrhizal	0.0136	0.0075	0.0328	0.0138	0.0031	0.0114	−0.0305	−0.0078
Ectomycorrhizal	0.0055	0.0054	0.0486	0.0463	0.0235	0.0530	−0.0788	−0.0074
Endophyte-Plant pathogen-Wood saprotroph	0.0070	0.0086	0.0003	0.0001	0.0025	0.0114	−0.0003	−0.0078
Plant pathogen	0.1481	0.1355	0.0325	0.0227	0.0341	0.0640	0.0110	0.2202

Note: SD, standard deviation; GRA, grazing; FEN, fencing.